We live in a world of viruses and our immune system is continuously fighting against them to keep us healthy and free of diseases. Our immune system is typically divided into two broad categories – the innate and the adaptive, both of which are required for defense against virus infection. The innate immune system, also known as the first line of antiviral defense, is kicked off very early during the infection. Whereas the adaptive immune system is activated at a later stage and is dependent on the innate immune responses. Therefore, the appropriate activation of the innate immunity is critical for the elimination of viruses from an organism. Type I interferon (IFN) system represents a key innate immune response mechanism against a wide range of viruses. Our cells are equipped with sensors, e.g. TLRs, RLRs, NLRs or cGAS/STING, which detect the incoming virus particles either outside or inside specific cellular compartments and trigger intracellular signaling pathways. These signaling pathways are responsible for induction of interferons, which protect the virus-infected, as well as the neighboring uninfected, cells by inducing hundreds of antiviral proteins.

In addition to the induction of antiviral genes, the virus-infected cells commit pre-mature suicide by triggering apoptotic cell death. We have reported that Interferon Regulatory Factor 3 (IRF3) is required for the virus-induced apoptotic cell death, which we named as RLR-induced IRF3 mediated Pathway of Apoptosis (RIPA) (Figure 1). IRF3 is a transcription factor, which gets activated by virus-induced signaling pathways and is essential for the induction of many antiviral genes, including IFN-β. In the uninfected cells, IRF3 remains inactive in the cytosol, upon activation by virus infection it gets translocated into the nucleus, where it binds to the promoters of the target genes. We have shown that specific cellular proteins, e.g. HDAC6, β-catenin and PKC-β are critical for the complete activity of IRF3. Our active investigation of the transcriptional signaling of IRF3 prompted us to investigate whether RIPA requires the transcriptional activity of IRF3. To address this, we used various genetically modified human and mouse cells, which were defective in specific signaling components. This series of experiments revealed that RIPA requires, in addition to some common proteins, a few additional pathway-specific components, such as several TRAF proteins. This led us to specifically investigate IRF3’s transcriptional activity in RIPA. Surprisingly, the mutants of IRF3, which were inactive in the transcriptional activity, could still trigger RIPA. This gave us strong indication that we had identified a new apoptotic signaling pathway, in which a new activity of IRF3 was required (Chattopadhyay et al, EMBO J, Cell Cycle, J Virol).

The obvious next generation question was how IRF3 triggers RIPA. To address this, we screened a series of IRF3 mutants for the transcriptional, RIPA...
Innate antiviral defense mechanisms of IRF3. Virus infection is recognized by the cytoplasmic cellular sensor RIG-1, which binds to viral double-stranded RNA and triggers two signaling branches, involving mitochondrial adaptor, IPS-1. In the transcriptional pathway, IRF3 is activated to induce antiviral genes. Whereas, in the RIPA branch, IRF3 is activated by a novel mechanism, LUBAC-induced ubiquitination, which triggers mitochondrial activation and apoptotic cell death. Both pathways contribute to the overall protection against viruses.

Our discovery of a new in vitro function of IRF3 raised the question whether RIPA protects against viral pathogenesis in vivo. To address this, we generated genetically modified (knock-in) mice, in which only RIPA, but not the transcriptional, branch of IRF3 was active. In our experimental knock-in mice, virus-induced antiviral genes were not produced; however, the RIPA branch was functional. We tested these mice against respiratory viral infection. Our results were quite striking and proved our hypothesis that the RIPA branch alone would prevent the lethal viral infection and protect the mice against respiratory diseases (Chattopadhyay et al Immunity 2016). We speculate that RIPA would protect against a variety of other viruses, which activate IRF3 to trigger apoptotic cell death. Although we have discovered RIPA in the context of viral infection, we believe that it can be tested in numerous diseases, in which cell death plays a critical role.

The new mouse model will provide a useful tool to specifically examine the impact of RIPA against not only other viral, but also bacterial, parasitic and non-microbial, diseases. RIPA-like pathways of IRF3 have been reported in other disease models. Human monocytes, when infected by a human retrovirus, HTLV1, trigger rapid apoptosis using a RIPA-like pathway. This protects the cells from HTLV1 infection. In a surprising study, RIPA-like pathway has been linked to alcoholic liver diseases (ALD). The hepatocytes, which are critical liver cells in ALD, undergo apoptotic cell death via RIPA-like activity of IRF3. Mice deficient in this pathway were resistant to ALD. Therefore, RIPA, which we thought would only function against viruses, also regulates non-viral diseases. Future studies would be required to investigate how RIPA is activated in other disease models, such as cancer, in which cellular apoptosis is a desired anti-cancer mechanism. Unregulated cell death causes inflammatory diseases; it will be interesting to study how RIPA functions in these disease conditions. In future, we plan to study how specific cells in an organism activate RIPA for a specific function and how RIPA can be targeted to design future therapeutics to treat human diseases. In collaboration with my previous mentor, Ganes Sen at Cleveland Clinic, and new collaboration with researchers at The University of Toledo, we will address these key physiological questions.

New Faculty

Normal epithelial cells have defense mechanisms against cancer

Saori Furuta, Ph.D.
Assistant Professor
Dept Biochem Cancer Biol

The number of new cases of cancer is 0.5 percent in men and women in the US. This figure seems staggering at first. But given that our body has 50 trillion cells and each cell could accumulate ~25 sporadic mutations in the genome per replication, we should realize that the cancer incidence is actually a lot less than what it could be. Furthermore, our cells are constantly exposed to carcinogens, mutagens, oxidative stress, UV radiation, etc. Then, why aren’t we getting more cancers than what we actually get?

The answer is that our body is equipped with inherent defense mechanisms against cancer. These include immunosurveillance, DNA damage repair, cell death, senescence and anti-oxidant mechanisms. Besides, normal epithelial cells utilize additional protective mechanisms against malignancy. First, epithelial cells are
surrounded by the basement membrane, the fibrous extracellular matrix (ECM) that filters out unwanted molecules, such as excessive growth factors and inflammatory cytokines. Second, cells carrying "Loser" mutations are eliminated by their surrounding "Winner" cells through a process termed "cell competition." Third, our laboratory previously reported that normal breast epithelial cells undergoing alveologenesis (i.e., mammary gland development during pregnancy) secrete a collection of factors that could selectively kill tumor cells, without affecting normal cells or subdue them into a dormant state (Furuta et al. 2011; Furuta et al. 2005). These mechanisms become less efficient as we grow older, making cells more susceptible to a stress that could trigger cancer initiation.

In our laboratory, we are currently examining the possibility of an additional mechanism by which normal breast epithelial cells defend themselves against breast cancer. We hypothesize that nitric oxide (NO), produced by normal breast epithelial cells in response to ECM, biochemically modulates (i.e., S-nitrosylate) cells and microenvironment to protect them against oxidative and fibrogenic stress. We then hypothesize that a reduction of NO removes such protection and deregulates fibrogenic signals that could alter ECM (i.e., stiffen ECM) and trigger cancer initiation. Stiffened and fibrous ECM is a predicative marker as well as a powerful driver of cancer initiation. Importantly, patients with chronic disorders (e.g., diabetes, obesity, aging and cardiovascular disease) succumb to reduction of NO because of oxidative impairment of NO-producing machinery, and reduced NO accounts for their increased fibrosis and cancer risks. It is possible that there is a close similarity in the pathogenesis of cancer and chronic disorders. We are utilizing high-resolution and atomic force microscopy, rhometry, 3D organotypic co-culture of primary cells as well as animal models to test our hypotheses. We are also planning to profile S-nitrosylated proteins in normal vs. cancerous breast cells by mass spectrometry and test the relevance of differential modification by mutagenesis. We hope that completion of this project will fundamentally advance our understanding of cancer initiation process and enable the development of earlier cancer detection and prevention methods.

From Lef: Samantha Metzer, Gang Ren, Saori Furuta

**COMLS Research Symposia**

The Internal Medicine Residency Program held the annual research symposia during the month of May. During the residents and fellows symposia, residents and fellows in the Department of Medicine presented various research projects that were also accepted for presentations at various regional and national meetings. 1st, 2nd, and 3rd place winners were chosen by faculty judging panels. The winner will receive a certificate along with a prize at the Department of Medicine Awards Ceremony which will be held at the Premier Banquet Facility on June 3rd, 2016.

**12th Annual Dr. Thomas Walsh Fellowship Research Symposium: May 4, 2016**

1st Place Winner ($500 Gift Card): Dr. Raja Thotakura (Gastroenterology), Interobserver Variability in the Diagnosis of Sessile Serrated Adenomas.

2nd Place Winner ($250 Gift Card): Dr. Mohammad Chowdhury (Cardiovascular Medicine), Importance of pre-operative right ventricular echocardiographic parameters in patients undergoing CABG.

3rd Place Winners ($100 Gift Card each):
Dr. Hadeel Altayib (Infectious Diseases), Asymptomatic Bacteruria in Renal Transplant, A Systemic Review.
and
Dr. Aahd Kubbara (Pulmonary-Critical Care) "Ventilator Associated Pneumonia: Looking for more
Culprits?

12th Annual Dr. Peter White Residents Research Symposium: May 11, 2016

1st Place Winner ($500 Gift Card): Dr. Mohammad El Zein, PGY 1, Learning Curve for Per-Oral Endoscopic Myotomy.

2nd Place Winner ($250 Gift Card): Dr. Madiha Fida, PGY2, Rapidly Progressive Dyspnea with Unexpected Autopsy Findings.

3rd Place Winner ($100 Gift Card): Dr. Mohammed Ruzieh, PGY2, Differential Outcomes to Cardiac Synchronization Therapy in Patients with Ischemic and Non-Ischemic Cardiomyopathy.

12th Annual Dr. William Sodeman Practice Based Learning, Quality Improvement & Patient Safety Symposium: May 25, 2016

1st Place Winners ($500 Gift Card): Tariq Hammad, MD-PGY 3, Komal Masood, MD-PGY-2, Abdelmoniem Moustafa, MD-PGY 1, Umar Shuaib, MD-PGY 1, Meghana Medavaram, MD-PGY-1, Jae Eun Lee, MD-PGY 1. Principal Investigator: Dr. Ammar Kayyali, The 4 M's Approach to Fall Prevention.

2nd Place Winners ($250 Gift Card): Mohammed Ruzieh, MD-PGY 2, Turki Alkully, MD-PGY 2, Khaled Shunnar, MD-PGY 1. Principal Investigator: Dr. Juan Jaume, Comparing Glycemic Control and Outcomes Between Old and New Insulin Infusion Protocols.

3rd Place Winners ($100 Gift Card): Sharifa Alhassan, MD-PGY 3, Yaseen Alastal, MD-PGY 3, Mohammed Ruzieh, MD PGY 2, Osama Dasa, MD-PGY 2, Brian Tasma, MD-PGY 1, Zaid Ammari, MD-PGY 1. Principal Investigator: Dr. Jodi Tinkel, Factors Affecting Enrollment in Cardiac Rehabilitation at UTMC.

Congratulations to all the winners on their well-deserved prizes. The Internal Medicine Residency Program is proud of the resident's accomplishments especially in the domain of research and scholastics. The Internal Medicine Residency Program takes pride in the initiatives that the Internal Medicine residents have taken and led in the areas of Quality Improvement and Patient Safety.

Clinical Research Snippets

Benefit and Harm of Dual Antiplatelet Therapy

By: Ankusk Moza, MD & Ehab Eltahawy, MD
Editors: Anand Mutgi, MD & Sadik Khuder, PhD

The optimal duration of dual antiplatelet therapy (DAPT) after percutaneous coronary intervention (PCI) and coronary stenting has been a matter of debate. Patients on longer duration of DAPT appear to have less frequent in-stent thrombosis but higher bleeding risks. This has led to the varying duration of treatment with DAPT after placing drug-eluting stents.

This month we review the results of the study by Yeh et al that addresses the optimal duration of DAPT. The authors use the DAPT study to build a risk prediction model using nine independent clinical factors that maximize the absolute risk vs. benefit for individual patients on a dual antiplatelet regimen. The goal of such a model is to help clinicians in decision-making based on baseline characteristics, which define individual risk of bleeding vs. ischemic events.
For the risk prediction model the authors assigned one point each for: myocardial infarction at presentation, prior myocardial infarction or PCI, diabetes, stent diameter less than 3 mm, smoking, and paclitaxel-eluting stent. They assigned two points each for history of CHF or ejection fraction less than 30 percent and presence of vein graft intervention. The authors assigned -1 point for age 65-75 years and -2 points for 75 years or older. A total score of < 2 was considered to be low risk for late thrombotic events and a score of 2 or greater was considered to be at high risk. In the high risk group, DAPT treatment reduced ischemic events significantly (5.75 vs. 2.7%) compared to the low score group (1.7% vs. 2.3%). Increase in bleeding risk was smaller in high-risk individuals (1.8% vs. 1.4%) compared with those at low risk (3% vs. 1.4%). The risk score generated by this model seems able to identify patients who would benefit from prolonged DAPT treatment without defining prolonged treatment. The model did not account for other variables including medication compliance, nonresponders to clopidogrel, and anemia. A major limitation is that in the DAPT trial, a significant number of patients had received early-generation drug-eluting stents (DES). Second- and third-generation DES designs have much lower risks for stent thrombosis, even with much shorter (< 6 months) courses of thienopyridine therapy.

Based on this study we feel that the risk prediction score is a useful tool that can be integrated into clinical decision making regarding the duration of DAPT, and that short-term use of DAPT is adequate in patients with low risk for stent thrombosis. This is consistent with the 2016 recommendations by the American College of cardiology and American Heart Association (ACC/AHA) suggesting 6 months of DAPT treatment is sufficient in patients at increased risk of bleeding.

**New Clinical Trials**

A Long-Term, Randomised, Double Blind, Placebo-Controlled Study to Determine the Effect of Albiglutide, when Added to Standard Blood Glucose Lowering Therapies, on Major Cardiovascular Events in Patients with Type 2 Diabetes Mellitus (HARMONY).

Dr. Hejeebu - Medicine

A Double Blind, Randomised, Placebo-Controlled Trial Evaluating Efficacy and Safety of Oral Nintedanib Treatment for at least 52 Weeks in Patients with 'Systemic Sclerosis associated Interstitial Lung Disease’ (SSc-ILD).

Dr. Kahaleh - Medicine

AE6134: A Randomized Phase III trial of Dabrafenib + Trametinib followed by Ipilimumab + Nivolumab at Progression vs. Ipilimumab + Nivolumab followed by Dabrafenib + Trametinib at Progression in Patients With Advanced BRAFV600 Mutant Melanoma.

Dr. Skeel - Medicine

**IRB Corner**

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